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L. Gott

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Memorandum

To: Marc F. Hult
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From: Edward M. Godsy
WRD, Western Region, Menlo Park, CA

Subject: Bacterial Sampling at St. Lewis Park, MN

In a microbiological sense, we are dealing with an enrichment culture in a more or less continuous flow system. This can be characterized as some form of continuous bacterial culture fed with a multiple substrate and inhabited by a flexible, mixed microbial population selected by the medium and process conditions. It is generally thought that the microbial communities in the formation are mostly contained in adherent films and that the growth substrate flows past them.

When a water sample is taken from a flowing well, we are actually counting those cells that are dislodged from the films and are entrained in the water moving towards the well bore.

One important assumption made is that all of the microbial types in the formation near the well will appear in the pumped water sample; therefore, any difference in the composition of the microbial flora between wells in the sampling area are suggestive of real difference in the quality of the ground water.

It is well known that microbes can decompose organic matter in anaerobic environments; these have been called anaerobic organotrophic eco systems. The rumen and gastrointestinal tracts of certain animals, flooded soils, sediments, sewage digestors, and ground waters are examples of these ecosystems. The transformations of organic materials under anaerobic conditions are varied but CO_2, CH_4, and H_2S are generally the end products. In these systems, methanogenic bacteria and sulfate reducing bacteria act as the terminal organisms in the food chain; therefore, for successful operation of the chain, complex organic materials must first be converted into simpler substances such as H_2, CO_2, and low molecular weight fatty acids, amino acids, etc., by the facultative anaerobic and obligate anaerobic bacteria.

Several other reductions can also occur in these environments. Reductions of nitrate to N_2O and N_2, reduction of hydrated ferric oxides to Fe^{+2}, and reduction of manganic oxide to Mn^{+2} are sometimes observed.

In order to do a complete reconnaissance for microbial activity at St. Louis Park several different types of analyses and experiments were performed. They were as follows:

1. VIABLE COUNTS
   Enumeration of the major physiological types of bacteria that would be expected in an anaerobic ecosystem.

   A. TOTAL AEROBIC BACTERIA
      Those bacteria capable of growth on aerobically prepared general medium which would include strict aerobes, facultative anaerobes, and denitrifying bacteria.

   B. TOTAL ANAEROBIC BACTERIA
      Those bacteria capable of growth on an anaerobically prepared general medium which would include facultative anaerobes of strict anaerobes.

   C. SULFATE REDUCING BACTERIA
      Those specialized bacteria that use sulfate as a terminal electron acceptor and are found only in areas of active anaerobic degradation of organic compounds.

   D. DENITRIFYING BACTERIA
      Those bacteria capable of growth in anaerobic environments using nitrate as the terminal electron acceptor.

   E. IRON REDUCING BACTERIA
      Those bacteria capable of reducing ferric and manganic oxides in anaerobic environments.

   F. METHANE BACTERIA
      Those bacteria capable of producing CH_4 from CO_2 and simple organic acids found only in areas of active anaerobic degradation of organic compounds.

2. DIRECT COUNT
   Direct microscopic enumeration of all bacteria in a water sample.

3. Survey of bacteria in the water samples capable of aerobic degradation of phenol, naphthalene, and a commercially available creosote.

4. The ability of the hydrocarbon phase from Well 13 to support aerobic growth when supplied as the sole source of both carbon and energy.

5. Qualitative analysis of dissolved gases on selected wells.

The numbers of the different physiological types of bacteria and direct counts do not show any significant differences between samples; however, as you approach the hydrocarbon pool in both the Drift and Platteville Aquifers the samples contain methane bacteria. This is indicative of active organic degradation with methane bacteria as the terminal member of the food chain. The presence of methane bacteria in the surface aquifer is probably due to the slow degradation of the peat deposits.

As you approach the hydrocarbon pool in Drift Aquifer, the populations also contain bacteria capable of degrading naphthalene. Also these samples contained dissolved CO_2 and CH_4.

In another experiment, 20 ml of the hydrocarbon fluid from W 13 was added to 50 ml of mineral salts medium. These flasks were then inoculated with $\sim 10^3$ cells/ml of a mixed bacterial culture acclimated for growth on polynuclear aromatic compounds. After seven days of aerobic growth at 30° C, the cell counts had increased to $\sim 10^7$ cells/ml. This indicates that at least some of the compounds in the hydrocarbon pool will support growth.

We now come to that point at which one would ask "What does it all mean?" Looking at the various data, it appears that in the Drift Aquifer active organic degradation is taking place between P 124 and W 117. The presence of bacteria capable of degrading naphthalene, the presence of dissolved CO_2 and CH_4, and the entire suite of bacteria, including methane bacteria, necessary to complete the conversion of the coal tar acids to CO_2 and CH_4, are present. The low numbers of bacteria are probably due to the low temperature of the water ($\sim$11°C).

In the Platteville Aquifer, there is not as much positive data, but the presence of methane bacteria in W 107 and W 18 would indicate that there is some organic degradation taking place.

This still leaves the following questions unanswered:

1. What compounds in the hydrocarbon fluid are degradable aerobically and anaerobically? Are they different?

2. What is the overall rate of conversion of the hydrocarbon fluid to methane and CO_2?

3. Are the organics being degraded between W 117 and P 124 coal tar acids or some other source of organics?

I believe these questions can be answered with a few appropriate experiments in my laboratory. It would depend on having the gas chromatographic equipment that has been ordered being set up and working as soon as possible.

I would like to make another trip to St. Louis Park in October to further characterize the bacterial populations in the water samples and possibly take some auger cores for bacteriological analysis. This could answer some questions regarding the areas of active degradation.

I will contact you regarding the October trip as soon as the experiments have been decided upon.

Mike

Edward M. Godsy

cc: Herman R. Feltz, WRD, Northeastern Region Headquarters
Reston, VA

TABLE 1

WELL	AEROBIC BACTERIA	ANAEROBIC BACTERIA (3 types of media)			SULFATE REDUC. BACT.	DENITRIFYING BACTERIA	IRON REDUCING BACTERIA	METHANE BACTERIA	DIRECT COUNT
P 23	9.3×10^4	9.3×10^3	2.3×10^3	9.3×10^3	ND	4.3×10^1	1.1×10^3	1.5×10^1	1.1×10^7
P 8	7.5×10^4	4.3×10^4	2.3×10^4	2.3×10^4	ND	9.3×10^2	1.1×10^3	4.3×10^1	9.2×10^6
W 2	2.3×10^5	9.3×10^1	2.3×10^2	4.3×10^2	4	9.3×10^1	9.3×10^1	ND	3.7×10^6
P 117	4.3×10^6	2.3×10^1	4.3×10^2	4.3×10^1	ND	4.3×10^3	4.3×10^2	ND	1.5×10^7
W 117	4.3×10^3	2.3×10^1	7.5×10^1	2.3×10^1	ND	9.3×10^1	4.3×10^1	2.3×10^1	4.9×10^6
P 14	1.5×10^4	2.1×10^2	7.5×10^3	9.3×10^4	ND	2.3×10^1	4.3×10^3	2.4×10^2	1.1×10^7
P 124	9.3×10^4	2.3×10^2	1.5×10^3	9.3×10^3	ND	4.3×10^3	1.5×10^2	4.3×10^1	1.1×10^7
W 13	9.3×10^2	2.3×10^1	2.3×10^2	4.3×10^4	ND	1.5×10^2	9.3×10^1	9	-
W 100	4.3×10^3	2.3×10^1	2.3×10^2	2.3×10^1	ND	2.3×10^1	9.3×10^1	ND	9.9×10^5
W 124	2.3×10^4	9.3×10^1	9.3×10^2	4.3×10^2	ND	4.3×10^2	4.3×10^1	ND	3.5×10^6
W 107	9.3×10^3	2.3×10^1	2.3×10^2	2.3×10^1	ND	4.3×10^1	2.3×10^1	2.3×10^1	6.0×10^6
W 18	2.3×10^3	2.3×10^2	2.3×10^2	1.5×10^2	ND	2.3×10^2	2.3×10^2	4.3×10^1	6.6×10^4
W 137	9.3×10^4	2.3×10^1	9.3×10^2	2.3×10^2	ND	4.3×10^1	4.3×10^1	ND	1.1×10^7

ND = None detected, <3/100 ml. No bacteria in 3 10 ml portions of the water sample.

TABLE 2

WELL	PHENOL	NAPHTHALENE	CREOSOTE	DISSOLVED CH_4 & CO_2
P 23	+	-	-	ND
P 8	+	-	-	ND
W 2	+	-	-	ND
P 117	+	+	-	ND
W 117	+	-	-	+
P 14	+	+	-	+
P 124	+	+	-	+
W 13	+	-	-	ND
W 100	+	-	-	ND
W 124	+	-	-	ND
W 107	+	-	-	ND
W 18	+	-	-	ND
W 137	+	+	-	ND

1.0 ml of water sample was inoculated in 50 ml of the mineral salts medium containing 300 mg/L of either phenol, naphthalene, or creosote. Samples were incubated at 30° C on a rotary shaker (~150 rpm).

ND = Not done, no obvious dissolved gases.